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We are working towards the goal of creating a bacterium that contains only the set of genes that are essential for life. Toward that end, we have continued to delete genes and gene clusters from the starting genome (*M. mycoides* JCVI-syn1.0). To date, the smallest viable genome with a reasonable doubling time is 778 kb. We have also made progress on the design and synthesis of a minimal genome. Construction of the initial design of minimized 1/8th genome molecules is complete, and assembly and transplantation experiments are in progress.

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J. Craig Venter™
INSTITUTE

Construction of a Bacterial Cell that Contains Only the Set of Essential Genes Necessary to Impart Life

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Report Number:	HR0011-12-C-0063.2
Reporting Period:	August 17, 2012 to November 16, 2012
Contract No.:	HR0011-12-C-0063
Performing Organization:	J. Craig Venter Institute 9704 medical Center Drive Rockville, MD 20850 USA
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Abstract

We are working towards the goal of creating a bacterium that contains only the set of genes that are essential for life. Toward that end, we have continued to delete genes and gene clusters from the starting genome (*M. mycoides* JCVI-syn1.0). To date, the smallest viable genome with a reasonable doubling time is 778 kb. We have also made progress on the design and synthesis of a minimal genome. Construction of the initial design of minimized 1/8th genome molecules is complete, and assembly and transplantation experiments are in progress.

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Summary

The goal of the project is to create a cell that contains only the set of genes that are essential for life under ideal laboratory conditions. We are working to minimize *Mycoplasma mycoides* JCVI-syn1.0 (the synthetic version of *Mycoplasma mycoides* subsp *capri*) using two approaches:

- Top Down: remove genes and clusters of genes one (or a few) at a time, proceeding only if the reduced strain is viable, with a reasonable growth rate
    - The genome has been reduced to 778 kb and found to be viable
    - Combinatorial assembly of eight 1/8th genome molecules has been demonstrated
        - Will allow simultaneous deletions to be made on each 1/8 molecule

- Bottom Up: design our best guess as to the content of a minimal genome and synthesize it from oligonucleotides
    - Synthesis of the initial design of the minimal 1/8th molecules has been completed
    - Assembly and transplantation experiments are underway
    - The first 1/8th genome molecule to be synthesized has been assembled into a full genome and transplanted
        - **The resultant genome (870 kb) was found to be viable.**
        - Strain grows very slowly, forming colonies in approximately 6 days
        - Data from the top-down strategy shows that one or two deleted areas could be causing the slow growth
        - A replacement 1/8th molecule with these two suspected deletions added back is being synthesized

Introduction

The primary goal of this research is to make a minimal bacterial cell. J. Craig Venter Institute (JCVI) will construct a new strain of the bacterium *Mycoplasma mycoides*, controlled by a genome that contains only essential genes. The minimal cell will (i) define the minimal set of genetic functions essential for life under ideal laboratory conditions, (ii) discover the set of genes of currently unknown function that are essential and to determine their functions, (iii) serve as a simple system for cell modeling, (iv) allow for modularization of the genes for each process in the cell (translation, replication, energy production, etc.) and to design a cell from those modules, and (v) allow more complex cells to be built by adding new functional modules.

The starting point for minimization is the synthetic genome *M. mycoides* JCVI-syn1.0. There are two approaches for minimization:

1. Top Down: Start with the full size viable *M. mycoides* JCVI-syn1.0 synthetic genome. Simultaneously remove genes and clusters of genes one (or a few) at a time from sections of the genome. Periodically re-test for viability by assembling the reduced genome sections combinatorially, and transplanting the genomes. Only proceed to the next step if the preceding construction is viable and the doubling time is approximately normal.

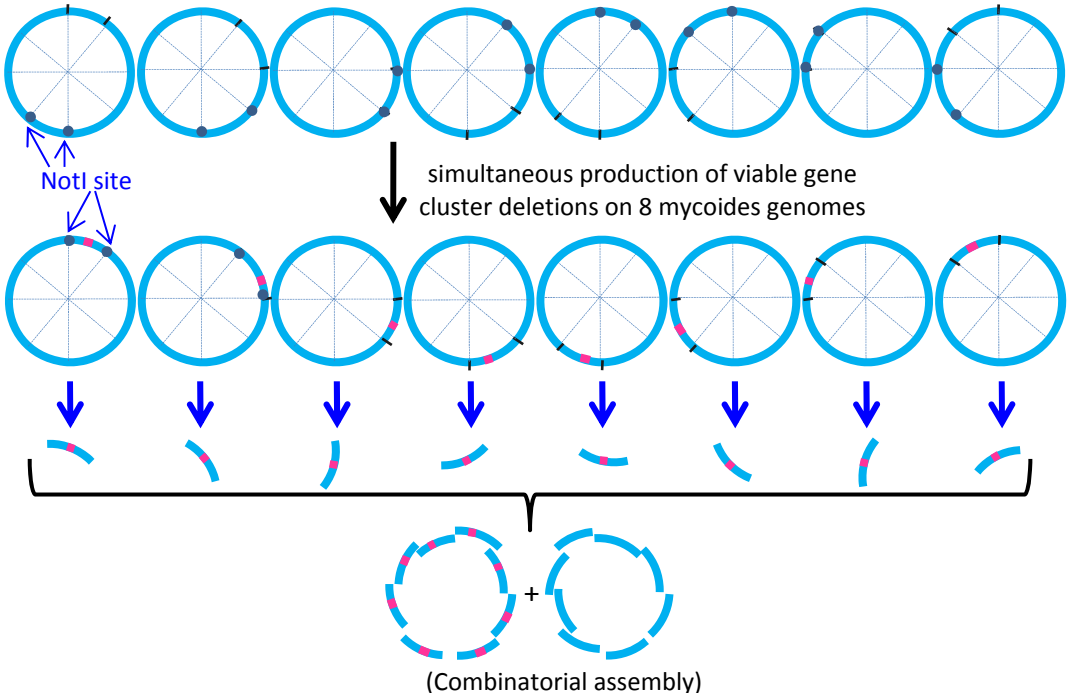
2. Bottom Up: Make our best guess as to the genetic and functional composition of a minimal genome, and then design and synthesize it from oligos. Craig Venter calls this the "Hail Mary" genome (HMG).

Methods, Assumptions and Procedures

TOP DOWN APPROACH

The previous approach was to make step-wise deletions of genes and gene segments using the TREC deletion method. This would allow steady progress to be made toward genome minimization. A modified approach has been recently developed and will be used going forward.

A combinatorial assembly method will be followed by the team. In this approach, viable gene cluster deletions will be generated simultaneously using TREC in sections of eight *M. mycoides* genomes. All eight reduced sections would be released by restriction, mixed with full length versions and assembled using homologous recombination in yeast. The resultant genomes will then be transplanted into recipient cells. A schematic representation of the approach is shown below:



BOTTOM UP APPROACH

The design of a minimal genome was previously reported. The design was separated into eight 1/8th genome molecules. Each of these 1/8th molecules will be assembled into designated areas of 7/8th genomes to create complete genomes. The reduced genomes will be transplanted to test for viability.

Results and Discussion

TOP DOWN APPROACH

Step-wise Deletion Approach

Targeted, stepwise deletion of genes and gene clusters predicted to be non-essential by transposon mutagenesis was continued, and progress to date is shown below:

	Size	Growth
M. mycoides wild type	1089 kb	++++
M. mycoides JCVI-syn 1.0	1079 kb	++++
M. mycoides JCVI-syn 1.0 – 6RM (17 genes, 17 kb)	1062 kb	++++
M. mycoides JCVI-syn 1.0 – 6RM (17 genes, 17 kb) – 6 IS (14 genes, 13 kb)	1049 kb	++++
M. mycoides JC syn1.0 – 6RM(12 genes, 17 kb) – 6 IS (12 genes, 9 kb) – ICE (44 genes, 71 kb)	980 kb	++++
M. mycoides JC syn1.0 – 6RM(17 genes, 17 kb) – 6 IS (14 genes, 13 kb) – 18 clusters (148 genes, 235 kb)	814 kb	++

Compared to the starting genome (*M. mycoides JC* syn1.0), the 814 kb genome has 179 genes and 265 kb removed from the genome.

In this reporting period, the team also conducted a large series of experiments aimed at testing deletions of gene clusters for viability:

- Deletion of 122 individual clusters was attempted
- 90 out of 114 genomes (about 79%) have been successfully transplanted; growth rates vary among the deleted strains
- The results from the cluster deletion experiments will be used in the next iterative design of the 1/8 molecules in the Bottom Up Approach

Combinatorial Approach

The combinatorial assembly approach has been successfully demonstrated. Seven reduced genome sections were mixed with eight parental genome sections and transformed into yeast:

	Section 1	Section 2	Section 3	Section 4	Section 5	Section 6	Section 7	Section 8	Possible Size
Reduced Genome Section	112 kb	67 kb	86 kb	NA	84 kb	93 kb	103 kb	98 kb	764 kb
Parental Genome Section	134 kb	121 kb	133 kb	123 kb	95 kb	119 kb	113 kb	111 kb	946 kb

Complete genomes were assembled via homologous recombination and transplanted into recipient cells. The efficiency of assembling a complete genome was > 80%. The possible 764 kb genome was not located from the combinatorial assembly experiment; however, viable genomes resulted from the combinatorial assembly, which demonstrates that the approach is valid. Colonies were genotyped using a multiplex PCR assay, and size confirmed by gel electrophoresis.

In an alternate experiment, six reduced sections (genome sections 1-3, and 5-8) and one parental section (section 4) were mixed, assembled by homologous recombination and transplanted. In this type of experiment, the resultant genome is will be the smallest possible molecule, but the chance that the transplanted genomes will be slow growing or non-viable is very high. Even though the reduced

sections had been previously confirmed as viable, once all of the reduced sections are combined the interaction of the deletions can lead to deleterious effects. Viable mycoplasma colonies with a reduced 778 kb genome were located and genotyped. The expected genome size was 764 kb, but two deletions in section number 8 were not in the transplanted clone. This could be because the yeast clone used to conduct the recombination happened to harbor more than one copy of the mycoplasma genome, and the deleted areas were recombined back into the genome. The colonies formed in approximately 3 days, representing a reasonable growth rate.

BOTTOM UP APPROACH

As previously reported, a minimal genome was designed by the project team. The minimal genome was designed in 1/8th genome molecules, so that smaller sections could be tested for viability. Since the last reporting period, all eight of the 1/8th genome sections were synthesized and sequence confirmed.

The initial 1/8th molecule to be synthesized (HM#2) has been assembled into a full molecule and transplanted. The genome was found to be viable, but displays a slow growth rate (colony formation takes 6 days). Information from deletions made in the Top Down Approach after the minimized genome was designed suggests that there are two viable deletions in HM#2 that cause slow growth. A new HM#2 is being synthesized with these two deletions added back into the sequence, in order to restore the growth rate to previous levels.

Examination of the remaining seven synthetic, minimal 1/8th molecules is in progress. They are in various stages of assembly into full genomes and transplantation.

Conclusions

Work has continued on the Top Down Approach to delete genes and gene clusters. Using the combinatorial assembly method will allow parallel work to make deletions around the genome possible. We expect that this will increase the speed and efficiency of this approach.

Testing of the minimal 1/8th genome molecules in the Bottom Up Approach is in progress.

Planned Activities for the Next Reporting Period

We will continue the effort to minimize the genome using targeted deletions and combinatorial assembly methods. We will also continue to test minimized 1/8th genome molecules for functionality. Sections that are found to be non-viable or slow growing will be redesigned and resynthesized.

Program Financial Status

	Planned Expend	Actual Expend (Cumulative to Date)	% Budget Completion	At Completion	Latest Revised Estimate	Remarks
Task 1	$305,646.19	$305,646.19	100.0%	$305,646.19	$305,646.19	Completed
Task 2	$869,742.81	$206,629.34	23.8%	N/A	$869,742.81	N/A
Cumulative	$1,175,389.00	$512,275.54	43.6%	N/A	$1,175,389.00	N/A

There is no management reserve or unallocated resources.

Based on the currently authorized work:

- Is current funding sufficient for the current fiscal year? Yes
- What is the next fiscal year funding requirement at current anticipated levels? $1,214,151.00
- Have you included in the report narrative any explanation of the above data and are they cross-referenced? Not applicable; current funding is sufficient for the current fiscal year.